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Activated T cells in the nasal mucosa of patients with grass-pollen allergy. A pilot study

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SUMMARY

Six patients with grass-pollen allergy were provoked with water-soluble grass pollen until a pronounced allergic reaction occurred. This was performed outside the grass-pollen season, and the allergen was administered on the edge of the inferior turbinate. Biopsies were taken both before provocation and during the reaction, 15–30 minutes after provocation.

The nasal population of immunohistochemically positive cells for HLA-DR, CD1, interleukin-2-receptor, IgE, CD4 and CD8 were studied. There was a marked increase of IL2-R-positive cells (activated T lymphocytes) in the nasal mucosa after provocation, whilst the other cell populations approximately remained unchanged (apart from a certain increase of IgE). The increase of activated T lymphocytes may imply that certain subsets of T cells play a role in the allergic response, and that the role of helper T cells very likely is much more complex than the regulation of mast cells and eosinophils. The concomitant presence of Langerhans' cells (CD1-positive) and activated T lymphocytes may indicate a possible association on site between an antigen-presenting cell and both effector as well as memory cells in allergic reactions.

INTRODUCTION

The Langerhans' cell has recently been reported to be present in the surface epithelium of the human nasal mucosa (Fokkens et al., 1989a, 1989b; Hellquist, 1990; Hellquist et al., 1991). The identification of Langerhans' cell requires either the demonstration of Birbeck granules by electron microscopy or immunohistochemical identification of the CD1-antigenic determinants on the cell surface

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of a dendritic cell (Murphy, 1985; Chu et al., 1987). Apart from having a capacity of binding IgE, the Langerhans' cell is capable of activating lymphocytes by releasing interleukin-1 (Murphy, 1985; Bruijnzeel-Koomen et al., 1989; Fokkens et al., 1989b; Ruco et al., 1989). Furthermore, the Langerhans' cell is known to be able of antigen presentation to T lymphocytes in contact-allergic reactions (Shelley & Juhlin, 1976; Stingl et al., 1978; Silberberg et al., 1979).

The aim of this study was to investigate the occurrence of Langerhans' cells and activated T lymphocytes in the nasal mucosa in allergic patients when provoked with the allergen. The presence of both Langerhans' cells and different subsets of T lymphocytes would open new theoretical aspects on allergic reactions, i.e. the Langerhans' cell releasing interleukin-1 after being in contact with the allergen and thus stimulating T lymphocytes to become activated T lymphocytes. The activated T cells may then undergo clonal expansion. This could imply that the T cells may play important roles in the nasal mucosa other than acute reactions such as the regulation of the synthesis of IgE.

MATERIAL AND METHODS

Nasal biopsies were taken from five patients with isolated grass-pollen allergy and from one patient with isolated mugwort-pollen allergy. None of the patients had taken any medication during the last two months before the study, which was undertaken well out of the pollen season. The first biopsy was taken from the inferior turbinate of the right nasal cavity. Water-soluble grass pollen and mugwort pollen (100,000 Units/ml; 0.2–0.4 ml) were then locally applied onto the inferior turbinate of the left nasal cavity until a macroscopical and objective reaction was achieved, i.e. swollen nasal mucosa and sneezing. In three of the patients repeated provocation had to be performed. The reactions occurred 15 to 30 minutes after administration and the second biopsy was then taken from this turbinate. The specimens were snap-frozen immediately.

The biopsy specimens were cut in a cryostat in as thin as possible sections. The sections were air dried at 25 °C for 16 hrs, and then fixed in acetone for 10 minutes at 37 °C. No inhibition of endogenous peroxidase was performed. The PAP method was used starting with blocking with normal rabbit serum (Dakopatts X 902) for 10 minutes (dilution 1:10). The primary incubation (Table 1) was performed at room temperature for 30 minutes. Rabbit anti-mouse immunoglobulin (Dakopatts P 260; dilution 1:10) was used for the secondary incubation. After application of the peroxidase-mouse antiperoxidase complex, the sections were stained with DAB, and then counterstained with Mayer's haematoxylin for 1 minute. The sections were then rinsed in tap water, dehydrated and mounted. The different antibodies used are shown in Table 1. The immunoreactivity was graded as negative (–) when no positive cells were found; + when one or two positive cells were found; ++ when several immunopositive cells were identified

Table 1. Characterization of the monoclonal antibodies.

antibody	source	reactivity
HLA-DR	Dakopatts	Human MHC Class II (to β chain of all DP, DQ and DR)
T6	Dakopatts	CD1 antigen
T4	Dakopatts	CD4 antigen
T8	Dakopatts	CD8 antigen
IL2-R	Dakopatts	interleukin-2 receptor
IgE	Dakopatts	IgE (IgE-bearing mast cells)

(more than 2, but less than 6 positive cells); and finally +++ when numerous positive cells were scattered throughout the mucosa (more than 5 positive cells). The grading was carried out using a ×40 objective, and the average score of 3 to 5 fields was calculated. The evaluation was performed on two occasions, and with the sections coded with regards to first and second biopsy, and concerning CD4 and CD8.

RESULTS

The results of the immunohistochemical investigation of the nasal mucosa before and after provocation with the allergen are shown in Table 2. The

Table 2. Immunoreactivity in the nasal mucosa of the six patients before and after provocation.

	reactivity			reactivity	
antibody	before	after	antibody	before	after
HLA-DR	+++	+++	T6	+	++
	+++	++		+	+
	+++	+++		+	+
	++	+++		++	+
	+++	+++		+	+++
	+++	+++		+	+
T4	++	+	T8	+++	++
	+	+		++	+++
	+	++		++	+++
	+	++		++	+
	+	+		+++	+++
	+	+		+	+
IL2-R	-	++	IgE	+	++
	-	+++		+	++
	-	+		-	+
	+	+		+	+
	-	+++		-	++
	+	+++		+	+++

expression of HLA-DR and CD1-positive cells in the surface epithelium did not change after provocation, i.e. there is no immediate change in the amount of surface cells capable of presenting the antigen to immunocompetent cells. Nor did the intraepithelial ratio CD4/CD8 change when the patient was provoked. There was a slight increase of the IgE-bearing mast cells in the lamina propria. A rather pronounced increase of IL2-R-positive T lymphocytes was observed (Figure 1), and it occurred in all cases but one (Table 2). There was no correlation between the severity of symptoms and the degree of increase of activated T lymphocytes.

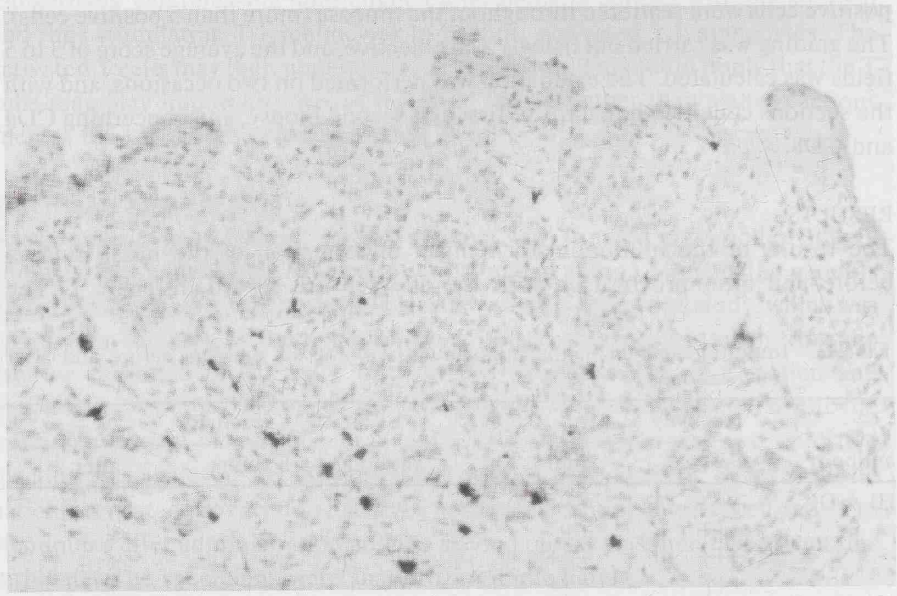

Figure 1. Photomicrograph of a nasal mucosa stained with interleukin-2 receptor showing several positive T-lymphocytes (Case 1; PAP, x 420).

DISCUSSION

The existence of Langerhans' cell in the nasal surface mucosa has now been confirmed in yet another study, and we now feel convinced that the Langerhans' cell is a normal constituent of the human nasal surface epithelium. Fokkens et al. (1989a) reported significantly more CD1-positive cells in the nasal mucosa of patients with isolated grass-pollen allergy during the season than before or after the season. However, we did not notice any increase in the number of Langerhans' cells after provocation. We believe that the results are not, however, in conflict with each other. They merely may reflect the fact that the Langerhans' cell, being a migrant cell, needs a certain amount of time to migrate to the site of

contact with the allergen. It may not be anticipated that this migration should take place and be completed within 15–30 minutes, on the contrary, it probably takes a day or two.

There was no change in the ratio between the CD4-positive T cells and the CD8-positive T cells. In an earlier study we have shown that T cells are much more common than B cells, and that cytotoxic/suppressor cells (CD8) are more common than helper/inducer cells (CD4) (Hellquist et al., in press). Furthermore, this study showed the CD4/CD8 ratio being similar in patients with allergy and in persons with non-specific nasal complaints, and comparable to that found in healthy subjects. In the present study, which included the lamina propria, CD8-positive cells outnumbered CD4-positive cells. A change of this ratio, if any, may occur but then probably later and, therefore, it could not be detected in this pilot study. Yet another possibility to be explored is that there may be a shift in the ratio between different subsets of T cells, whilst the total amount of CD4-positive cells then could remain relatively intact.

The possible immediate increase of IgE-bearing mast cells is difficult to explain satisfactorily. Due to technical difficulties in evaluating some of the detected IgE may not have been attached to mast cells, but merely produced. The increase is expected, but not to occur so quickly. The designation of the study with the six patients subjected to a strong provocation until a macroscopical and physiological reaction was noticed, may account for the results, and the test may simulate a reaction that normally comes first after longer exposure to the allergen.

A marked immediate increase of lymphocytes expressing the receptor for interleukin-2 (activated T lymphocytes) was observed in the lamina propria. Contrary to the migration of Langerhans' cells the activation of T lymphocytes is a rather rapid process. However, in vitro stimulation of CD45-R-positive lymphocytes by phytohaemagglutinin has revealed that the expression of IL-2 receptor appears quickly, but still first after approximately 15 hours (Akbar et al., 1989). On the other hand, it is known that high-affinity receptors for interleukin-2 are absent on resting T cells, but appear within hours of activation (Dinarello & Mier, 1987). In vivo stimulation by eg. interleukin-1 may show a similar course of events. However, the immediate response noticed in the present study needs further investigation. A proper statistical analysis is necessary, but also investigating the possibility of other synergistic stimuli (eg. different interleukins and interferons), and finally a comparison with non-allergic persons is indicated. An increase of activated T lymphocytes has already been reported in patients with allergic rhinitis (Holm et al., 1990).

The possible importance of the increase of activated T lymphocytes can be outlined as follows. In mice, helper-T-cell clones (CD4-positive) fall into two main groups (Mosmann and Coffman, 1989), namely TH_1 cells (synthesizing

IL-2, interferon-gamma, and lymphotoxin) and TH_2 cells (synthesizing IL-4, IL-5, and probably IL-6). The TH_1 cell itself is stimulated by interleukin-1 (which may be synthesized by Langerhans' cells) and Class II MHC antigen. If stimulated by different interferons (INF-alpha, beta and gamma) as well as by Tumour Necrosis Factor-alpha and beta (TNF-alpha and beta) the activated T lymphocyte ($CD4^+$, $IL2\text{-}R^+$) may through clonal expansion develop to memory TH as well as to effector TH cells. Similarly, CD8-positive T cells may be activated and express IL2-receptor. The activated T cells can produce IL2 as well as TNF-beta. After clonal expansion these T cells ($CD8^+$ origin) may develop to memory cells, but also to Activated Cytotoxic T Lymphocytes (CTL). CTL can, under the influence of TNF-beta and TNF-alpha, act directly on a target cell. Intriguingly, in vitro lymphocyte proliferation has been shown to be partially inhibited by anti-IL2 antibodies, and completely inhibited by anti-IL2- receptor antibodies (Tanaka et al., 1990). It seems as though human T cell clones do not fall into the TH_1- and TH_2-type subsets as described in mice (Quint et al., 1989). However, another – and to a certain extent similar – system may well exist as the CD4 "helper" population can be subdivided into $CD4^+2H4^+4B4^-$ cells which are "suppressor-inducers", and $CD4^+2H4^-4B4^+$ cells which are true helper cells (Morimoto et al., 1985; Takeuchi et al., 1987; Salmon et al., 1988).

The observations in the present study indicate a possible, but important, association between nasal Langerhans' cells and activated T cells in allergic reactions. The presence of activated T cells also makes it possible that the nasal mucosa contains memory T cells. In this context it is important to recall that virgin T cells are non-reactive to recall antigen and poor in helping B-cell differentiation whilst memory T cells can recall antigen and enhance Ig production (Byrne et al., 1988). Thus, there may be other possible pathways concerning primarily the late phase in allergic reactions, a sequence of interreactions between subsets of helper T cells and cytokines.

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